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Odd-numbered perfluorocarboxylates predominate over perfluorooctanoic acid in serum samples from Japan, Korea and Vietnam

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Abstract

Perfluorooctanoic acid (PFOA) has recently attracted attention as a potential health risk following environmental contamination. However, information detailing exposure to perfluorinated carboxylic acids (PFCAs) other than PFOA is limited. We measured the concentrations of PFCAs (from perfluorohexanoic acid to perfluorotetradecanoic acid) in serum samples obtained from patients in Japan (Sendai, Takayama, Kyoto and Osaka) between 2002 and 2009, Korea (Busan and Seoul) between 1994 and 2008 and Vietnam (Hanoi) in 2007/2008. Total PFCAs levels (geometric mean) were increased from 8.9 ng mL^{-1} to 10.3 ng mL^{-1} in Japan; from 7.0 ng mL^{-1} to 9.2 ng mL^{-1} in Korea; and were estimated at 4.7 ng mL^{-1} in Vietnam. PFCAs of greater length than PFOA were significantly increased in Sendai, Takayama and Kyoto, Japan, and levels of long-chain PFCAs exceeded PFOA levels in serum. Among these PFCAs, perfluoroundecanoic acid (PFUnDA) was the predominant component (28.5%), followed by perfluorononanoic acid (PFNA 17.5%), perfluorodecanoic acid (PFDA 7.9%), perfluorotridecanoic acid (PFTrDA 6.1%) and perfluorododecanoic acid (PFDoDA 1.8%). Odd-numbered PFCAs (PFNA, PFUnDA and PFTrDA) were also observed in Korea and Vietnam and their presence increased significantly in Korea between 1994 and 2007/2008. The proportion of long-chain PFCAs in serum was relatively high compared to reports in Western countries. Further investigations into the sources and exposure routes are needed to predict the future trajectory of these serum PFCA levels.

Key words: perfluorocarboxylate; perfluorooctanoic acid; serum; temporal trend; East Asia

Abbreviations

PFCAs: perfluorinated carboxylic acids

PFOS: perfluorooctane sulfonate

PFOA: perfluorooctanoic acid

PFHxA: perfluorohexanoic acid

PFHpA: perfluoroheptanoic acid

PFNA: perfluorononanoic acid

PFDA: perfluorodecanoic acid

PFUnDA: perfluoroundecanoic acid

PFDoDA: perfluorododecanoic acid

PFTrDA: perfluorotridecanoic acid

PFTeDA: perfluorotetradecanoic acid

IDLs: instrumental detection limits

MDLs: method detection limits

RSD: relative standard deviation

SD: standard deviation

GM: geometric mean

GSD: geometric standard deviation

63 **1. Introduction**

64 Perfluorinated compounds such as perfluorooctane sulfonate (PFOS) and
65 perfluorooctanoic acid (PFOA) have recently attracted attention owing to widespread
66 contamination of the environment, wildlife and humans by these chemicals (Houde et
67 al., 2006). In 2002, after 50 years of production, 3M Company phased out their
68 manufacture of PFOS (Renner, 2001). PFOA is considered to be a major component of
69 perfluorocarboxylates (PFCAs) emission. However, in Japan, PFCA emissions consisted
70 of not only PFOA but also perfluorononanoic acid (PFNA) and perfluoroundecanoic
71 acid (PFUnDA) (of which 25 and 7 metric tons, respectively, were emitted in 2000)
72 (Prevedouros et al., 2006). These odd-numbered PFCAs (PFNA, PFUnDA and
73 perfluorotridecanoic acid (PFTrDA)) were detected at higher concentrations in samples
74 from local wildlife than similar even-numbered PFCAs (PFOA, perfluorodecanoic acid
75 (PFDA) and perfluorododecanoic acid (PFDoDA), respectively) (Furdui et al., 2008).
76 Although studies using human samples from Western countries showed that PFOA was
77 the most prevalent (followed by PFNA, PFDA and PFUnDA) (Haug et al., 2009;
78 Joensen et al., 2009; Kato et al., 2009), our previous study of Japanese women in the
79 Miyagi prefecture showed that PFNA and PFUnDA (average: 2.8 and 5.4 ng mL^{-1},
80 respectively) were found at broadly similar serum concentrations to PFOA (average: 4.9
81 ng mL^{-1}) (Kärrman et al., 2009).

82 PFCAs with longer chains than PFOA have higher bio-concentration factors
83 suggesting persistency in the environment (Martin et al., 2003). Temporal trends in
84 serum levels after 2002 showed no apparent decline of PFNA, PFDA or PFUnDA in
85 Norway (Haug et al., 2009), although serum levels of PFOA and PFOS both decreased
86 in the United States, Norway and Japan (Harada and Koizumi, 2009; Harada et al.,

2010; Haug et al., 2009; Olsen et al., 2008). These findings suggest a possibility that the origin and source of exposure to long-chain PFCAs could differ from those of PFOA and PFOS.

In the present study, we investigated current serum concentrations of PFCAs in three Asian countries (Japan, Korea and Vietnam). We selected the cities of Busan and Seoul in Korea because they are comparably urban and industrialized to Osaka, Japan. To confirm the temporal trends in Japan and Korea, we used archived historical serum samples stored in the human specimen bank (Koizumi et al., 2009; Koizumi et al., 2005). Hanoi in Vietnam was selected to evaluate the development of PFCA contamination following recent industrialization.

2. Material and methods

2.1. Experimental design and study population

To evaluate geographical differences and temporal trends in Asian countries, we compared 521 samples collected from Japan (Sendai, Takayama, Kyoto and Osaka) between 2002 and 2009; Korea (Busan and Seoul) between 1994 and 2008; and in Hanoi, Vietnam between 2007 and 2008. Samples from Sendai and Takayama in 2008, Osaka, Busan, Seoul and Hanoi are identical to a previous analysis of PFOS and PFOA (Harada et al., 2010; Kärrman et al., 2009). A total of 521 serum samples with information on donor age, sex and residential history (>5 yr in each area) were selected from the archived samples in Kyoto Human Specimen Bank (Koizumi et al., 2009; Koizumi et al., 2005) (Table 1). Serum was separated from cellular components and stored at -30 °C until analysis.

The study population in Osaka and Kyoto consisted of residents that had been

intensely exposed to PFOA from a local industrial source (the fluoropolymer manufacturer, Daikin Company) (Harada et al., 2004, 2007, 2010; Kärrman et al., 2009; Niisoe et al., 2010). In contrast, there is no known potential industrial source of PFCAs that would affect sample populations in the other cities studied.

For historical comparisons, samples were selected so that age and gender were matched among time points, except for Busan in 2000 and Osaka (Table 1).

The research protocol for the present study was reviewed and approved by the Ethics Committee of the Kyoto University Graduate School of Medicine on 14 November 2003 (E25).

2.2. Reagents

Ammonium acetate (purity: >99% by HPLC) was purchased from Aldrich (Steinheim, Germany). Acetonitrile (LC-MS grade) and water (distilled LC-MS grade) were obtained from Kanto Chemicals (Tokyo, Japan). Acetic acid and benzyl bromide were purchased from Wako pure chemicals (Osaka, Japan). Mixture of native PFCAs, $^{13}C_4$-labeled PFOA and $^{13}C_5$-labeled PFNA were obtained from Wellington Laboratories (Guelph, Ontario, Canada).

2.3. Determination of PFCAs in serum

Perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and perfluorotetradecanoic acid (PFTeDA) were analyzed. Serum samples were subjected to a clean-up procedure using a dispersive carbon method described by Powley et al. (2005). Briefly, the serum samples (0.5 mL, except for Korean samples between 1994 and 2000, which were 0.25 mL) together with

an internal standard ($^{13}C_4$-PFOA, 1 ng) were extracted with 5 mL of acetonitrile, followed by centrifugation at 1600 × *g* for 15 min. The supernatants were transferred into new tubes with 25 mg of ENVI-Carb and 50 µL of acetic acid, and the solutions were mixed by vortexing for 30 s. After centrifugation at 1600 × *g* for 15 min, the extracts were dried under a nitrogen stream. The residue was then re-dissolved in 100 µl of 100 mM benzyl bromide acetone containing the recovery performance standard $^{13}C_5$-PFNA (1 ng) for 1 hour at 80 °C and transferred to an autosampler vial. Extracts were analyzed using gas chromatography-mass spectrometry (Agilent 6890GC/5973MSD, Agilent Technologies Japan, Ltd., Tokyo, Japan) in electron impact ionization mode using single ion monitoring. PFCA benzyl esters were separated on a DB-5MS column (30 m length, 0.25 mm i.d., 1 µm film thickness) with a helium carrier gas. Split-less injections (1 µl) were performed with the injector set at 220 °C, and the split was opened after 1.5 min. The initial oven temperature was 70 °C for 2 min, ramped at 20 °C min^{-1} to 100 C°, and then at 30 °C min^{-1} to 280 °C. Ion fragments ($[M]^+$) were monitored and used as quantification ions (Table 2).

Instrumental detection limits (IDL) were defined as the mass of analyte producing a peak with a signal-to-noise ratio of 3, and ranged from 1 pg (PFTeDA) to 0.25 pg (other PFCAs) (Table 2). Since blank samples (0.5 mL distilled water) contain no detectable concentrations, the method detection limits (MDL) value was considered to be equal to the IDL corresponding to 0.2 ng mL^{-1} for PFTeDA and 0.025 ng mL^{-1} for other PFCAs (Table 2).

2.4. Quality assurance

Quantification was performed using an internal standard method with the external

159 standards dissolved in 10% methanol in water. $^{13}C_4$-PFOA was used as the internal
160 standard for PFCAs. $^{13}C_5$-PFNA was used to calculate a recovery rate of $^{13}C_4$-PFOA.
161 All samples were quantified using a seven-point calibration curve with a relative
162 standard deviation (RSD) of the relative response factors <15% for all compounds. The
163 recoveries were evaluated by five replicate fortifications (fortified by 10 times the
164 original concentration of serum) of a human serum sample with low contamination
165 (Table 2). The procedural blank levels were evaluated in duplicate for 11 samples each
166 using 0.5 mL distilled water.

167 Using the above method, we reanalyzed 361 samples originally tested in a previous
168 study by HPLC-MS/MS (Harada et al., 2010; Kärrman et al., 2009). The reanalyzed
169 samples showed 5.14±11.60 ng mL^{-1} for PFOA, which equates to 101.7% of the levels
170 obtained in the previous study (5.05±11.16 ng mL^{-1}, p=0.478 by paired t-test). Pearson's
171 correlation coefficient, r and slope were 0.9882 and 1.128, respectively (p<0.0001).
172 Levels (mean±SD) of PFHpA, PFNA, PFDA and PFUnDA in Osaka in 2004 were also
173 confirmed in this study (HPLC-MS/MS vs GC-MS: 0.26±0.14 ng mL^{-1} vs 0.24±0.09 ng
174 mL^{-1}, 6.68±1.78 ng mL^{-1} vs 6.16±1.91 ng mL^{-1}, 2.55±0.99 ng mL^{-1} vs 2.74±1.32 ng
175 mL^{-1}, 5.80±2.13 ng mL^{-1} vs 5.12±2.69 ng mL^{-1}, respectively; p>0.05 by paired t-test).
176 RSDs of difference between methods were 33.1%, 9.8%, 13.6% and 11.5% for PFHpA,
177 PFNA, PFDA and PFUnDA, respectively and average RSD was 17.0%.

178 To assess potential interlaboratory difference in analysis, NIST standard reference
179 material (SRM) 1957 was analyzed (Table 2). The values from PFHpA to PFUnDA
180 were comparable to those from interlaboratory comparison exercises (Lindstrom et al.,
181 2009; Keller et al., 2010).

182 Mean recovery rate (RSD) of $^{13}C_4$-PFOA in 521 samples was 96.5% (8.8%). To

evaluate possible matrix effect in serum sample, we further analyzed 100 samples extracts fortified with 1 ng of PFHpA, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA standards. Recoveries of fortified standards were 98.7%, 104.6%, 102.0%, 97.2%, 102.2% and 96.3% for PFHpA, PFNA, PFDA, PFUnDA, PFDoDA and PFTrDA, respectively. It is therefore considered that there was no substantial suppression or enhancement of target ions, if any.

2.5. Statistical analysis

All statistical analyses were carried out using the JMP software (Version 4; SAS Institute Inc., Cary, NC). Values of $p<0.05$ were considered to indicate statistical significance. Concentrations of less than the detection limit were all approximated to 'half of detection limit' for statistical analyses. Serum levels of PFCAs were assumed to distribute lognormally because the serum levels of PFCAs in the samples displayed right-skewed patterns and geometric means were comparable to medians. Statistical analyses were conducted after logarithmic transformation of the serum concentrations. Differences between mean values were tested by Tukey-Kramer's honestly significant difference (HSD) test after ANOVA. Correlation was tested by Spearman's rank correlation coefficient (ρ). Factor analysis was used to transform a number of contaminants into a smaller number of potential factors of sources. Factor analysis was conducted *via* correlation matrix. In essence, the factor analysis is a model which presumes the existence of a smaller set of factors that can reproduce exactly the correlation in the larger set of variable. To achieve this goal, the linier combinations of factors (i.e., principal component) will be generated in such a manner that each

207 composite variate will account a smaller portion of the total variation i.e., variance.
208 Eigenvalues of a principal component is a measure how much this principal component
209 can account for the variation and eigenvector indicates an associated set of coefficients
210 with a principal component for each factor. Eigenvectors were employed through the
211 analysis when eigenvalues were close to or greater than 1 which means its eigenvector
212 can account the equivalent of one or more original variables. Normalized varimax
213 rotation (an orthogonal rotation of the factor axes) was applied to these eigenvectors to
214 simplify them into a few variables with high correlations.
215
216 **3. Results**
217 *3.1. Temporal changes in PFCA concentrations in Japan*
218 The descriptive statistics for PFCAs are presented in Table 3. Most samples
219 contained PFOA, PFNA, PFDA, PFUnDA and PFTrDA at both time points. No samples
220 contained PFHxA and PFTeDA at concentrations above MDL. PFHpA levels were
221 significantly decreased in all sampling sites in Japan between 2002/2004 and 2008/2009
222 ($p<0.05$ by Student's *t*-test). PFOA was relatively high in Osaka and Kyoto although
223 levels of this compound nevertheless significantly decreased in this period ($p<0.05$ by
224 Student's *t*-test). In Sendai and Takayama, PFOA levels also decreased but this
225 difference was not statistically significant. In contrast, PFCAs longer than PFOA
226 showed significant increases in Sendai, Takayama and Kyoto with few exceptions.
227 Among these PFCAs, PFUnDA was the predominant component, followed by PFNA,
228 PFDA, PFTrDA and PFDoDA. These odd-numbered PFCAs (i.e. PFUnDA, PFNA and
229 PFTrDA) were detected at higher concentrations than neighboring, even-numbered
230 PFCAs (PFDA and PFDoDA).

231 In Osaka, levels of PFNA, PFDA and PFUnDA, as with PFOA, significantly
232 decreased from 2004 to 2008. PFDoDA and PFTrDA levels did not change. Among four
233 sampling sites in 2008/2009, Osaka and Kyoto had higher PFOA, PFNA and PFDA
234 levels than Sendai and Takayama ($p<0.05$ by Tukey's HSD test) but PFUnDA, PFDoDA
235 and PFTrDA showed no regional differences ($p>0.05$ by ANOVA).
236 As a consequence of the increase in long-chain PFCAs, the proportion of PFOA in the
237 total PFCA content became less than 50% in all locations except Osaka.
238
239 *3.2. Temporal trends in the serum concentrations of PFCAs in Korea*
240 The PFCA concentrations in the serum samples collected in Busan and Seoul
241 between 1994 and 2008 are shown in Table 4. As is the case with Japan, PFOA, PFNA,
242 PFDA, PFUnDA and PFTrDA were frequently detected in 2007/2008. PFHxA and
243 PFTeDA were not detected in any samples at concentrations above MDL. In agreement
244 with the previous report by Harada et al. (2010), PFOA levels were stable from 1994 to
245 2008 in Busan and Seoul ($p>0.05$ by ANOVA). In contrast, odd-numbered PFCAs
246 (PFNA, PFUnDA and PFTrDA) were significantly increased during this period ($p<0.05$
247 by Tukey's HSD test or Student's *t*-test). The PFCA levels had the following order of
248 prevalence in 1994: PFOA>PFNA~PFUnDA>PFDA>PFTrDA>PFHpA~PFDoDA.
249 However, by 2007/2008 the order had changed to:
250 PFOA>PFUnDA>PFNA>PFDA~PFTrDA>PFDoDA>PFHpA. Between 1994 and
251 2007/2008, total PFCA levels were significantly increased by 1.31- and 1.53-fold in
252 Busan and Seoul, respectively ($p<0.05$ by Tukey's HSD test or Student's *t*-test).
253 Samples from Busan contained higher concentrations of PFHpA, PFOA, PFNA, PFDA,
254 PFUnDA and PFTrDA than did those from Seoul in both 1994 and 2007/2008 ($p<0.05$

255 by Student's *t*-test).

256

257 *3.3. PFCAs concentrations in Hanoi, Vietnam in 2008-2009*

258 PFOA, PFNA, PFDA and PFUnDA were detected in all samples, and PFDoDA and
259 PFTrDA were also detected, albeit less frequently (Table 5). PFHxA, PFHpA and
260 PFTeDA were not detected in any samples from Hanoi. The concentration of PFUnDA
261 was highest among the PFCAs studied, followed by PFNA, PFDA, PFOA, PFTrDA and
262 PFDoDA. The proportion of PFOA relative to total PFCAs was only 12.9%.

263

264 *3.4. Correlations among PFCA levels and factor analysis*

265 Correlation coefficients among PFCAs in 521 samples are listed in Table 6. PFHpA
266 was relatively less correlated with other PFCAs, except for PFOA (ρ=0.398). PFOA also
267 significantly correlated with PFNA and PFDA (ρ coefficient >0.5) but was less well
268 correlated with PFUnDA, PFDoDA and PFTrDA. In general, PFCA concentrations
269 indicated a strong correlation between PFCAs of similar (i.e. adjacent) chain length.

270 To delineate potential patterns in the data, PFCA concentrations were examined
271 using factor analysis. The contributions of factors 1 and 2 to the total variance were
272 49.72% and 19.40% (with an eigenvalue >1), respectively (Table 7). After varimax
273 rotation, the first factor indicated a higher eigenvector for longer-chain PFCAs than
274 PFNA. The second factor had a more positive eigenvector for shorter-chain PFCAs than
275 PFDA. Since there is a point source of PFCAs in both Osaka and Kyoto, we evaluated
276 whether this predominant source may perturb the results of the factor analysis.
277 Eliminating Osaka and Kyoto samples, however, did not alter a correlation matrix
278 among PFCAs with changes in eigenvalues being less than 5% (data not shown),

indicating that the dominant point source had no substantial influence on the interpretation of factor 1 and factor 2.

Factor 1 is characterized by PFUnDA dominance (factor loading: 0.858) and another by PFOA dominance (0.819), respectively. This characteristic pattern indicates fingerprints of PFCAs sources in Asia. Temporal transition of factor scores is demonstrated by score plots shown in Supplemental Fig. 1. In sampling sites in Japan and Korea (except for Osaka), centers of score plot moved rightwards and downwards, indicating that the factor 1 score increased and factor 2 score decreased during these periods. Mean factor scores of each sampling site are also shown in Table 7. In Japan, factor 1 scores significantly increased from 2002/2003 to 2008/2009 ($p<0.05$ by Student's *t*-test), except for Osaka which already had a high factor 1 score (0.92) in 2004. This increase in factor 1 scores was also observed in Busan and Seoul from 1994 to 2007/2008 ($p<0.05$ by Tukey's HSD test or Student's *t*-test). Although the factor 1 score in Hanoi was lower than those in other sites in 2007–2009, it surpassed scores in Sendai and Kyoto in 2002/2003 and in Busan and Seoul in 1994. Contrary to factor 1, factor 2 scores in all sampling sites in Japan significantly declined between 2002/2004 and 2008/2009 ($p<0.05$ by Student's *t*-test) and also in Busan and Seoul from 1994 to 2007/2008 ($p<0.05$ by Tukey's HSD test or Student's *t*-test). Factor 2 in Hanoi was the lowest among all sampling sites.

4. Discussion

In the present study, we uncovered two major fingerprints (factor 1 and factor 2) by analyzing serum samples from three countries in East Asia. Characteristic PFCA composition was observed for odd-numbered PFCAs such as PFUnDA and PFTrDA

303 with residual PFDoDA and PFDA, which can correspond to factor 1. Even in
304 populations exposed to low levels of PFOA, notably Hanoi, PFUnDA showed
305 substantial serum levels. Moreover, levels of those PFCAs with longer chain lengths
306 than PFOA were significantly elevated in Japan and Korea in recent years. In the
307 late-2000s, consequently, long-chain PFCA levels exceeded PFOA levels in most
308 sampling sites. This finding suggests an emergence of specific sources of exposure in
309 East Asia.

310 In several countries, serum PFOA has reportedly decreased (Harada et al., 2010;
311 Olsen et al., 2008). In contrast, PFCAs of longer chain lengths than PFOA were
312 frequently detected in serum samples in this study. Total levels of long-chain PFCAs
313 were comparable to or greater than PFOA levels (except in Osaka) and showed trends
314 towards increases in Japan and Korea. Correlation between PFOA and long-chain
315 PFCAs was not strong which suggests that the sources of long-chain PFCA
316 contamination have different exposure route than PFOA. Indeed, factor analysis
317 demonstrated two major factors as sources of PFCAs. The first factor had loading on
318 longer-chain PFCAs than PFOA and the second factor on PFHpA, PFOA and PFNA.
319 Temporal trends of these factors were opposite and contamination derived from factor 1
320 might be expected to emerge in around a decade. This transition of factor scores was
321 similar in Japan, Korea and Hanoi. Contamination derived from factor 1 may have been
322 prevailing in East Asian countries.

323 Among long-chain PFCAs, odd-numbered PFCAs accounted for the major
324 proportion. Serum or blood levels of PFCAs reported from populations in China, Sri
325 Lanka, Australia, Norway, Sweden, Denmark, Poland, Belgium, Spain and USA are
326 summarized in Table 8 (Ericson et al., 2007; Falandysz et al., 2006; Guruge et al., 2005;

Haug et al., 2009; Joensen et al., 2009; Kärrman et al., 2006; Kuklenyik et al., 2004; Pan et al., 2010; Roosens et al., 2010; Toms et al., 2009). The PFCA composition in our current study, which was characterized by a large proportion of PFUnA, was apparently different from Western countries (Table 8). Although PFOA levels in these countries were comparable, long-chain PFCAs were not major components in Western countries, except for Antwerp, Belgium and Atlanta, USA. Therefore, this composition can be considered as a clear fingerprint for East Asian countries and is implicated in the origination of factor 1.

However, their source remains unclear due to insufficient monitoring data of PFCAs. Interestingly, a review by Prevedouros et al. (2006) indicated that PFNA has been manufactured in Japan *via* oxidation of fluorotelomer olefins together with PFUnDA and PFTrDA. Industrial application of these odd-numbered PFCAs, namely Surflon S-111, might contribute to the East Asian-specific pattern of serum body burdens. The temporal increase in long-chain PFCAs warrants further investigations of the sources and exposure routes to assist in predicting future changes in the serum levels of these contaminants.

In this study, there was a limitation in chemical analysis. $^{13}C_4$-PFOA was used for internal standard for PFCAs (C_7-C_{14}). Chemical properties of PFCAs may, however, be different even though they have similar structures. Matrix effects also might affect quantification of PFCAs other than PFOA. Thus it is logically possible that recovery rates of $^{13}C_4$-PFOA might be extensively deviated from those of other PFCAs. Neverthelss, such a possibility is unlikely because recovery rates of PFCAs were higher than 90% and RSD were within 10%, indicating that there was no substantial difference in recoveries among PFCAs in this method. Furthermore, a good agreement of results

in SRM analysis by interlaboratory comparisons assured that our analytical method in this study is sound. Collectively, these findings consistently support that analytical method in this study was sufficiently qualified.

Recent epidemiological investigations have raised concern regarding developmental effects of PFOA on children (Steenland et al., 2010). In contrast, few studies have been conducted on the effects of PFCAs of different chain length. Even though PFCAs have similar structure, their chemical properties and biological activity are likely different. In several *in vitro* studies, long-chain PFCAs caused biological responses at lower doses than PFOA (Liao et al., 2009; Matsubara et al., 2006; Upham et al., 1998). The toxicokinetics of long-chain PFCAs are also unclear, especially in humans. These uncertainties necessitate more comprehensive toxicological studies on PFCAs.

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482 **Figure captions**

483 **Supplemental Fig. 1.**

484 Plot of first- and second factor scores of 521 samples in Osaka (A), Kyoto (B),
485 Takayama (C), Sendai (D), Busan (E) and Seoul and Hanoi (F). Overall, 50% of the
486 values locate within the boundary circles for each sampling site and time period.

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Table 1
Study area and study population

Sampling site	Population (x10^3)	Latitude and longitude	Year	n	(%female	Age[a]	(range)
Japan							
Sendai	1,031	38°17'04" N 140°55'46" E	2008	50	(100)	37.5±9.44	(21-53)
	1,023	-	2003	50	(100)	36.6±10.1	(20-59)
Takayama	94 (65)[b]	36°08'13" N 137°15'16" E	2008	50	(100)	40.5±4.78	(29-49)
	67	-	2003	50	(100)	39.9±4.5	(31-45)
Kyoto	1,466	35°01'18" N 135°46'38" E	2009	30	(50)	33.2±14.7	(21-68)
	1,469	-	2002	30	(50)	35.4±11.3	(21-58)
Osaka	2,652	34°45'31" N 135°31'52" E	2008	50	(100)	45.9±8.92^{A*}	(30-63)
	2,619	-	2004	10	(100)	60.9±6.3^{B}	(49-69)
Korea							
Busan	3,711	35°14'39" N 129°05'54" E	2008	35	(100)	40.1±6.44^{A*}	(18-49)
	3,732	-	2000	30	(100)	35.4±4.27^{B}	(28–45)
	3,961	-	1994	39	(100)	42.3±4.65^{A}	(34-52)
Seoul	10,421	37°27'52" N 127°01'56" E	2007	36	(100)	34.5±8.24	(20-54)
	10,798	-	1994	24	(100)	38.0±7.41	(24-51)
Vietnam							
Hanoi	6,232	21°00'08" N 105°49'50" E	2007-2008	37	(100)	30.2±5.76	(20-40)

* Means of age with different letters differed significantly ($p<0.05$ by Tukey's HSD test). For example, the letters A and B indicate that the corresponding values differ significantly at $p<0.05$.

a Data are presented as mean ± standard deviation.

b Takayama city merged with neighboring cities in 2005. Numbers in paretheses denote populations areas corresponding to those used in 2003.

Table 2
Recovery, detection limits and QA for PFCA analysis in human serum samples

Compound	Quantification (confirmation)	Recovery and (reproducibility) % (RSD%)[a] (n=5)	Instrument detection limit [b] (pg)	detection limit[c] (ng mL^{-1})	SRM1957[d] (ng mL^{-1})
PFHxA	404 (385)	92.2 (8.41)	0.25	0.05	<0.05
PFHpA	454 (435)	94.5 (4.12)	0.25	0.05	0.27
PFOA	504 (485)	101.7 (6.99)	0.25	0.05	4.77
$^{13}C_4$ PFOA	508 (489)	102.8 (5.47)	-	-	-
PFNA	554 (535)	97.4 (7.61)	0.25	0.05	0.96
$^{13}C_5$ PFNA	559 (540)	-	-	-	-
PFDA	604 (585)	91.9 (8.63)	0.25	0.05	0.26
PFUnDA	654 (635)	94.1 (7.22)	0.25	0.05	0.16
PFDoDA	704 (685)	95.7 (4.87)	0.5	0.1	<0.1
PFTrDA	754 (735)	98.6 (9.41)	0.5	0.1	<0.1
PFTeDA	785 (786)	92.4 (8.18)	1	0.2	<0.2

[a] RSD: relative standard deviation
[b] 1 µl injection
[c] 0.5 mL serum sample
[d] 0.5 mL serum sample of NIST SRM 1957 was analyzed.

Table 3
Serum concentrations of PFCAs in Japan

Sampling site	Year	n		Concentration (ng mL^{-1}) PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	ΣPFCAs
Sendai	2008	50	GM(GSD)	0.06(2.17)*	2.44(1.56)	1.80(1.40)*	0.72(1.46)*	3.00(1.59)*	0.17(1.99)*	0.60(2.00)*	9.13(1.41)*
			range	<0.05-0.37	0.85-6.05	0.90-3.58	0.31-1.58	1.15-8.08	<0.1-0.52	<0.1-1.43	3.81-17.52
			detection%	58	100	100	100	100	82	96	
	2003	50	GM(GSD)	0.15(3.75)	2.65(1.61)	1.01(1.85)	0.52(1.71)	1.68(1.75)	0.10(1.85)	0.31(2.12)	6.92(1.51)
			range	<0.05-1.25	0.87-7.59	0.21-4.94	0.09-1.57	0.32-5.70	<0.1-0.37	<0.1-1.13	2.74-17.94
			detection%	72	100	100	100	100	58	100	
Takayama	2008	50	GM(GSD)	0.04(2.29)*	2.51(1.84)	1.78(1.42)*	0.85(1.51)*	3.12(1.51)	0.20(2.15)*	0.60(2.66)	9.87(1.39)
			range	<0.05-0.49	0.82-11.25	1.01-4.50	0.26-2.68	1.28-7.13	<0.1-0.61	<0.1-2.46	5.44-22.09
			detection%	38	100	100	100	100	82	94	
	2003	50	GM(GSD)	0.11(2.35)	3.19(1.62)	1.30(1.73)	0.65(1.63)	2.74(1.60)	0.14(1.88)	0.55(1.72)	9.18(1.49)
			range	<0.05-1.72	1.36-20.28	0.64-9.88	0.18-2.26	0.77-7.81	<0.1-0.51	0.16-1.98	4.49-37.04
			detection%	88	100	100	100	100	80	100	
Kyoto	2009	30	GM(GSD)	0.11(1.98)*	5.28(1.57)*	2.78(1.42)*	1.10(1.45)	3.20(1.64)*	0.24(1.87)*	0.45(1.57)*	13.67(1.42)
			range	<0.05-0.31	2.60-16.52	1.34-4.40	0.60-2.25	1.20-11.26	<0.1-0.99	0.22-1.15	6.60-26.81
			detection%	96.7	100	100	100	100	93.3	100	
	2002	30	GM(GSD)	0.23(1.89)	7.12(1.54)	2.09(1.67)	0.91(1.66)	1.89(1.65)	0.12(2.04)	0.31(1.83)	12.98(1.52)
			range	0.08-1.25	2.69-19.64	0.81-5.37	0.35-2.54	0.72-5.44	<0.1-0.37	<0.1-1.00	5.38-33.75
			detection%	100	100	100	100	100	66.7	96.7	
Osaka	2008	50	GM(GSD)	0.07(3.11)*	13.46(1.79)*	3.54(1.62)*	1.11(1.60)*	3.05(1.73)*	0.16(2.55)	0.52(2.62)	23.08(1.64)*
			range	<0.05-1.11	5.59-201.68	0.85-14.57	0.36-2.80	1.01-8.79	<0.1-0.75	<0.1-1.95	10.77-220.07
			detection%	48	100	100	100	100	68	94	
	2004	10	GM(GSD)	0.21(2.00)	29.54(1.29)	6.41(1.38)	2.38(1.48)	5.45(1.46)	0.25(2.28)	0.44(2.40)	45.42(1.27)
			range	0.05-0.45	20.60-45.20	3.07-9.22	1.41-4.17	3.19-9.01	<0.1-0.51	<0.1-1.02	31.67-65.57
			detection%	100	100	100	100	100	90	90	

GM: Geometric mean; GSD: Geometric standard deviation
* GMs between time points are significantly different in each sampling site ($p<0.05$ by Student's t test after log transformation).

Table 4
Serum concentrations of PFCAs in Korea

Sampling site	Year	n		Concentration (ng mL^{-1}) PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	ΣPFCAs
Busan	2008	35	GM(GSD)	0.04(1.92)*^{A}	4.67(1.40)	1.91(1.45)*^{A}	0.91(1.38)	2.91(1.54)*^{A}	0.20(2.03)*^{AB}	0.94(1.92)*^{A}	11.87(1.38)*^{A}
			range	<0.05-0.16	2.77-9.80	1.02-3.89	0.44-1.76	1.03-7.62	<0.1-0.81	<0.1-2.74	6.70-24.13
			detection%	40	100	100	100	100	85.7	97.1	
	2000	30	GM(GSD)	0.10(1.59)^{B}	3.69(1.47)	1.77(1.41)^{A}	0.84(1.45)	2.06(1.66)^{AB}	0.14(1.61)^{A}	0.72(1.67)^{A}	9.58(1.39)^{B}
			range	<0.1-0.28	1.19-7.33	0.89-3.61	0.32-1.47	0.58-3.95	<0.20-0.39	<0.20-1.73	4.31-16.00
			detection%	30	100	100	100	100	33.3	100	
	1994	39	GM(GSD)	0.10(1.58)^{B}	4.11(1.43)	1.35(1.96)^{B}	0.89(1.65)	1.37(2.81)^{B}	0.11(1.61)^{B}	0.36(2.90)^{B}	9.05(1.46)^{B}
			range	<0.1-0.32	1.72-9.63	<0.10-5.20	0.25-2.98	<0.20-13.16	<0.20-1.03	0.10-2.89	4.08-32.50
			detection%	35.9	100	97.4	100	92.3	7.7	69.2	
Seoul	2007	36	GM(GSD)	0.03(1.48)	2.29(1.34)	1.13(1.32)*	0.58(1.38)	2.18(1.48)*	0.12(2.03)	0.59(2.10)*	7.10(1.35)*
			range	<0.05-0.12	1.22-4.64	0.74-2.01	0.32-1.00	1.10-5.62	<0.10-0.38	<0.10-1.54	3.94-12.55
			detection%	13.9	100	100	100	100	66.7	97.2	
	1994	24	GM(GSD)	0.08(1.00)	2.09(1.54)	0.65(2.01)	0.45(2.06)	0.54(3.89)	0.10(1.26)	0.16(2.40)	4.63(1.49)
			range	<0.1	0.89-4.09	<0.1-1.73	<0.1-1.18	<0.20-3.59	<0.20-0.31	<0.20-1.08	2.56-10.69
			detection%	0	100	95.8	95.8	70.8	4.2	25	

GM: Geometric mean; GSD: Geometric standard deviation
* GMs among different time points are significantly different in each sampling sites ($p<0.05$ by Student's t test or Tukey's HSD test after log transformation). Alphabetic suffix was used for comparisons among three groups. For example, the letters A and B indicate that the corresponding values differ significantly at $p<0.05$., while A and AB or AB and B indicated that the corresponding values do

Table 5
Serum concentrations of PFCAs in Hanoi, Vietnam

Sampling site	Year	n		Concentration (ng mL^{-1}) PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	ΣPFCAs
Hanoi	2007-2008	37	GM(GSD)	0.03(1.00)	0.61(1.55)	0.89(1.47)	0.82(1.67)	1.55(1.53)	0.09(1.85)	0.36(3.38)	4.73(1.38)
			range	<0.05	0.20-1.43	0.35-1.65	0.19-2.03	0.57-3.95	<0.10-0.26	<0.10-1.99	2.58-9.43
			detection%	0	100	100	100	100	51.4	86.5	

GM: Geometric mean; GSD: Geometric standard deviation

Table 6
Correlation between different chain length PFCAs

Combination		ρ	p value
PFOA	PFHpA	0.398	<0.001
PFNA	PFHpA	0.223	<0.001
PFNA	PFOA	0.734	<0.001
PFDA	PFHpA	0.165	<0.001
PFDA	PFOA	0.534	<0.001
PFDA	PFNA	0.727	<0.001
PFUnDA	PFHpA	0.019	0.660
PFUnDA	PFOA	0.323	<0.001
PFUnDA	PFNA	0.646	<0.001
PFUnDA	PFDA	0.689	<0.001
PFDoDA	PFHpA	0.055	0.208
PFDoDA	PFOA	0.235	<0.001
PFDoDA	PFNA	0.462	<0.001
PFDoDA	PFDA	0.563	<0.001
PFDoDA	PFUnDA	0.740	<0.001
PFTrDA	PFHpA	-0.117	0.008
PFTrDA	PFOA	0.063	0.151
PFTrDA	PFNA	0.264	<0.001
PFTrDA	PFDA	0.360	<0.001
PFTrDA	PFUnDA	0.552	<0.001
PFTrDA	PFDoDA	0.471	<0.001

ρ: Spearman's rank correlation coefficient

Table 7
Factor analysis among PFCAs

	Initial solution		Varimax rotated	
	F1	F2	F1	F2
Eigenvalue	3.48	1.36		
Contribution (%)	49.72	19.40		
Eigenvector				
PFHpA	0.092	0.618	-0.198	0.713
PFOA	0.365	0.480	0.327	0.819
PFNA	0.474	0.179	0.673	0.610
PFDA	0.469	0.036	0.745	0.459
PFUnDA	0.446	-0.230	0.858	0.168
PFDoDA	0.374	-0.266	0.760	0.066
PFTrDA	0.274	-0.481	0.719	-0.244
Factor score (mean±standard deviation)				
Sendai	2008		0.31±0.78*	-0.41±0.67*
	2003		-0.84±0.90	0.17±0.92
Takayama	2008		0.50±0.67*	-0.49±0.85*
	2003		-0.10±0.76	0.02±0.81
Kyoto	2009		0.44±0.68*	0.68±0.52*
	2002		-0.46±0.85	1.29±0.50
Osaka	2008		0.91±1.03	1.17±0.78*
	2004		0.92±0.71	2.42±0.48
Busan	2008		$0.68\pm0.67^{\dagger A}$	$-0.28\pm0.56^{\dagger A}$
	2000		0.06 ± 0.61^{B}	0.15 ± 0.46^{A}
	1994		-0.48 ± 0.95^{C}	0.42 ± 0.63^{B}
Seoul	2007		$0.02\pm0.69^{\dagger A}$	$-0.92\pm0.36^{\dagger A}$
	1994		-1.49 ± 0.90^{B}	-0.22 ± 0.48^{B}
Hanoi	2007-2008		-0.27±0.74	-1.48±0.65

F1: 1st factor; F2: 2nd factor
* Means between time points are significantly different ($p<0.05$ by Student's t test)

† Means among different time points are significantly different ($p<0.05$ by Student's t test or Tukey's HSD test). For example, the letters A and B indicate that the corresponding values differ significantly at $p<0.05$., while A and A or B and B indicated that the corresponding values do not differ significantly.

Table 8
Comparison of serum or whole blood concentrations of PFCAs with reported data

Sampling site	Year	n	Sex		Sample	Concentration (ng mL^{-1}) PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTrDA	reference
Japan													
Sendai	2008	50	F	median	serum	0.07	2.36	1.82	0.73	2.97	0.19	0.74	This study
	2003	50	F	median	serum	0.22	2.59	1.07	0.55	1.79	0.11	0.37	This study
Takayama	2008	50	F	median	serum	<0.05	2.08	1.72	0.80	3.11	0.24	0.77	This study
	2003	50	F	median	serum	0.13	3.21	1.29	0.69	2.69	0.15	0.56	This study
Kyoto	2009	15/15	M/F	median	serum	0.11	5.52	2.69	1.01	3.15	0.25	0.45	This study
	2002	15/15	M/F	median	serum	0.23	7.20	2.15	0.90	1.72	0.14	0.30	This study
Osaka	2008	50	F	median	serum	<0.05	12.80	3.32	1.10	2.98	0.19	0.72	This study
	2004	10	F	median	serum	0.23	28.90	6.87	2.53	5.83	0.35	0.51	This study
Korea Busan	2008	35	F	median	serum	<0.05	4.64	1.98	0.92	3.00	0.22	0.92	This study
	2000	30	F	median	serum	<0.1	3.98	1.92	0.87	2.27	<0.2	0.76	This study
	1994	39	F	median	serum	<0.1	3.98	1.28	0.94	1.82	<0.20	0.49	This study
Seoul	2007	36	F	median	serum	<0.05	2.21	1.11	0.57	2.37	0.14	0.74	This study
	1994	24	F	median	serum	<0.1	2.31	0.76	0.50	0.89	<0.20	<0.20	This study
Vietnam Hanoi	2007-2008	37	F	median	serum	<0.05	0.63	0.91	0.85	1.58	0.11	0.65	This study
Norway	2006	>20	M	median	serum	0.078	2.7	0.55	0.22	0.14	<0.05	0.071	Haug et al., 2009
Sri Lanka Colombo	2003	10	M	median	serum	0.146	9.32	0.299	0.18	0.186	0.015	-	Guruge et al., 2005
China Ningbo	2006-2008	8/12	M/F	median	pooled serum	<0.1	3.28	0.984	0.718	0.917	<0.18	-	Pan et al., 2010
Spain Catalonia	2002-2007	24/24	M/F	median	whole blood	<0.78	1.65	0.41	0.24	0.2	-	-	Ericson et al., 2007
Poland Gdańsk	2003	10/5	M/F	median	whole blood	0.086	2.8	0.49	0.17	0.078	0.012	-	Falandysz et al., 2006
Belgium Antwerp	2002-2005	182	F		pooled serum	-	3.18	2.41	1.86	-	-	-	Roosens et al., 2010
Australia Queensland	2006-2007	42/42	M/F	mean	pooled serum	-	6.4	0.8	0.29	-	-	-	Toms et al., 2009
Denmark Copenhagen	2003	105	M	median	serum	0.2	4.9	0.8	0.9	0.1	0.08	<0.1	Joensen et al., 2009
Sweden Stockholm	1997-2000	40/26	M/F	median	whole blood	-	2.5	0.3	0.2	0.2	-	-	Karrman et al., 2006
Atlanta USA	2003	10/10	M/F	median	serum	-	4.35	2.35	0.35	0.7	-	-	Kuklenyik et al., 2004

Supplemental Figure 1